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Coniolepiota spongodes (*Agaricaceae*, *Basidiomycota*) in Bangladesh and China

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Abstract — The genus *Coniolepiota* is recorded for the first time from Bangladesh and China. *Coniolepiota spongodes* is characterized by its gray lilac to purple floccose basidiomata and relatively small smooth ellipsoid to oblong basidiospores. A detailed morphological description, photographs and line drawings, with confirmation of the phylogenetic position of newly reported taxon are provided herein.

Key words — *Agaricales*, Asian fungus, ITS-nrLSU, morphology, new record

Introduction

Coniolepiota Vellinga, a genus of family *Agaricaceae* recently described from tropical Asia, harbors a single relatively well-known species, *C. spongodes* (Vellinga et al. 2011). During studies of the higher fungi (Hosen & Ge 2011; Hosen et al. 2013), collections of *C. spongodes* were made for the first time from Bangladesh. A study of the Bangladesh and Chinese materials is provided here.

Materials & methods

Morphological studies

Collections were made along roadsides in forests of *Shorea robusta* Gaertn. (*Dipterocarpaceae*) in Bangladesh, and from tropical China. Fresh specimens were described and photographed in the field. Dried specimens were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China (KUN-HKAS), and in the Herbarium of Agarics Flora, Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh (SHAF).

Microscopic observations were made in 5% aqueous KOH, and structures drawn by free hand. The notations '(n/m/p)' indicate that the measurements were made on 'n' basidiospores from 'm' basidiomata of 'p' collections with a minimum of 20 basidiospores per basidioma. Dimensions of basidiospores are presented in the following form

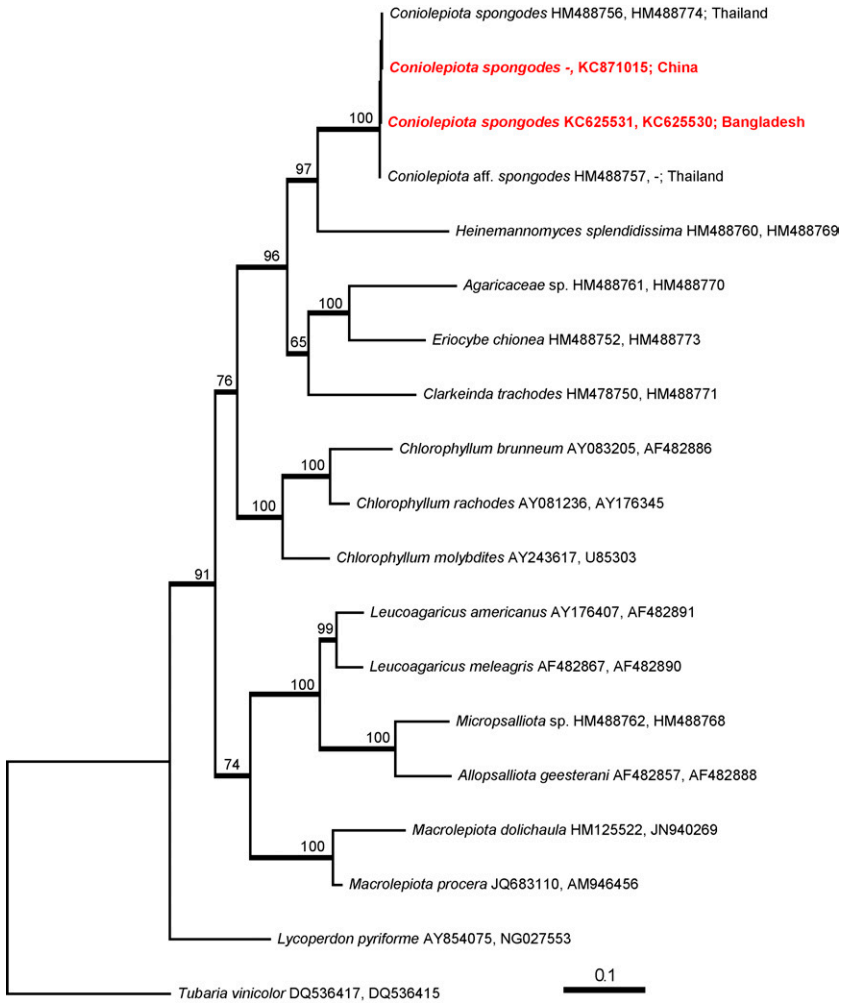

FIGURE 1. A phylogenetic tree generated from the combined ITS–nrLSU dataset of *Agaricaceae* using maximum likelihood (ML) method. Posterior probabilities from Bayesian inference (BI) (>0.97) are indicated as thick branches and bootstrap values derived from ML (BS>60%) are shown above the branches at nodes. Newly generated sequences for *Coniolepiota spongodes* are highlighted in boldface with red color. GenBank accessions ITS and nrLSU are provided after the species name and unavailable sequence is marked as hyphen sign.

(a–)b–c(–d); in which 'b–c' contains at least 90% of the measured values and extreme values 'a' and 'd' are set in parentheses. $Q_m = Q \pm SD$; Q refers to the ratio of individual spore, Q_m refers to the average Q of basidiospores, and SD is the standard deviation.

Molecular procedures and phylogenetic analysis

The protocol for DNA extraction followed those of Doyle & Doyle (1987). ITS1/ITS4 (White et al. 1090) and LROR/LR5 (Vilgalys & Hester 1990) primer pairs were used for the amplification of internal transcribed spacer (ITS) and the large subunit nuclear ribosomal RNA (nrLSU) regions, respectively. PCR amplification, sequencing and sequence alignment, Maximum Likelihood (ML) and Bayesian Inference (BI) methods followed those in Hosen et al. (2013). Phylogenetic analysis was conducted on the combined dataset of the ITS and the nrLSU.

Molecular results

Two sequences were newly generated in this study and are deposited in GenBank. The combined ITS-nrLSU dataset consisted of 1689 nucleotide sites (including gaps). In this dataset, 1116 characters were constant, while 573 characters were variable, of which 211 were parsimony uninformative and 362 were parsimony informative. Parsimony analysis resulted in one most parsimonious tree of 1329 steps, with Consistency Index (CI) = 0.634, Retention Index (RI) = 0.590 and Rescaled Consistency Index (RC) = 0.374. Tree topologies obtained from both ML and BI methods of phylogenetic analysis were almost identical. Sequences from collection of *C. spongodes* made from Bangladesh fall in a highly supported clade with those described from northern Thailand (Fig. 1).

Taxonomy

Coniolepiota spongodes (Berk. & Broome) Vellinga, Mycologia 103: 502, 2011.

Figs 2, 3

Basidiomata solitary, medium-sized. Pileus 40–80 mm broad, plano-convex to applanate with slightly inflexed margin, covered with lilac to purple-gray powdery velar warts at young stage which remain in some areas especially in the center, but in outer zone sometimes disappearing, showing the pallid to whitish background; warts thick at center, becoming spread out towards margin, sometimes with powdery warts hanging over the margin. Lamellae 14 × 4 mm, free and remote from the stipe, crowded with lamellulae, pallid cream to pale yellowish. Stipe 25–50 × 6–10 mm, central, cylindrical, usually slightly tapering upward, glabrous and pallid white at apex but the remaining parts covered with powdery lilac warts as on pileus, base slightly swollen and covered with whitish mycelium, hollow. Annulus present, thin membranous and easily detachable. Context 7 mm thick at the center of the pileus, dull white to slightly pale purplish.

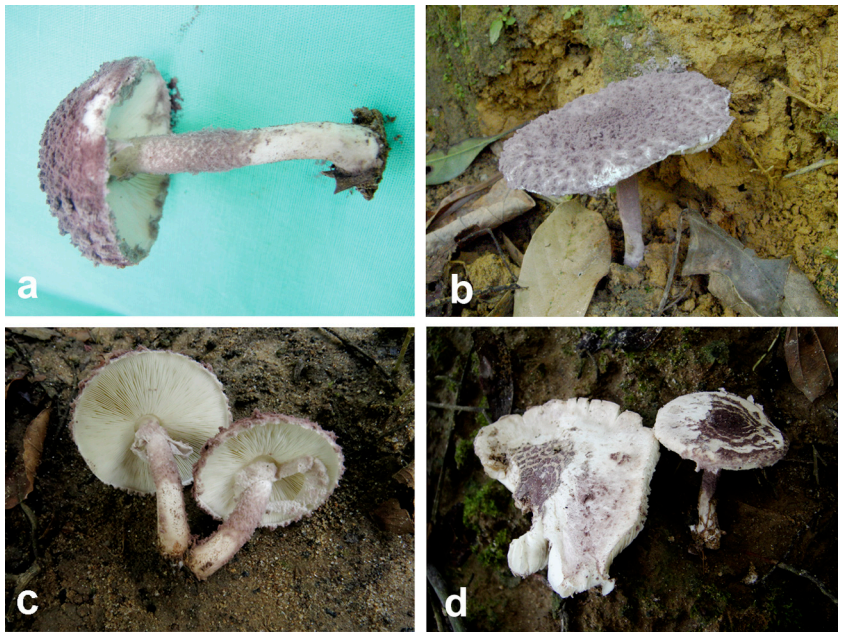

Figure 2. *Coniolepiota spongodes*. a–b. Basidiomata show gray lilac velar coverings on the surface of pileus and stipe. c. Hymenophore showing free, crowded, pallid to cream-white lamellae with powdery warts hanging over the margin. d. Gray lilac velar coverings disappearing from the surface of the pileus and stipe at maturity and/or handing. (a = HKAS 77576; b = HKAS 60246; c = SHAF 5; d = HKAS 77574). Photos: a, c, d, Md. Iqbal Hosen; b, Zai-Wei Ge.

Basidiospores [80/4/4] 4–5(–6) × (2.8–)3–3.2(–3.5) µm [Q = 1.43–1.57, Q_m = 1.52 ± 0.127], ellipsoid to somewhat oblong, hyaline to pale yellowish, dextrinoid, inamyloid, nearly rounded apex, smooth, slightly thick-walled, without germ pore. Basidia 12–20 × 5–7 µm, cylindro-clavate to clavate, hyaline to pale yellowish in H_2O, thin-walled, tetrasporic but occasionally 2- or 3-spored. Cheilocystidia 20–26 × 6–9 µm, clavate to clavulate, some cylindro-clavate, hyaline, thin-walled, smooth, without encrustations, not abundant, scattered and sometimes hard to find. Pleurocystidia not observed. Pileus covering a powdery layer made up of irregularly shaped cylindrical cells, three dimensional (T-shaped), some anastomosing (H-shaped), individual elements 20–50 × 4–10 µm, hyaline, smooth, without incrusting pigments, refractive connecting points often present at the septate portion or near to the ending points of the pileal hyphae. Stipe covering similar to those of pileus but refractive connecting points is less common. Clamp connections absent in all tissues.

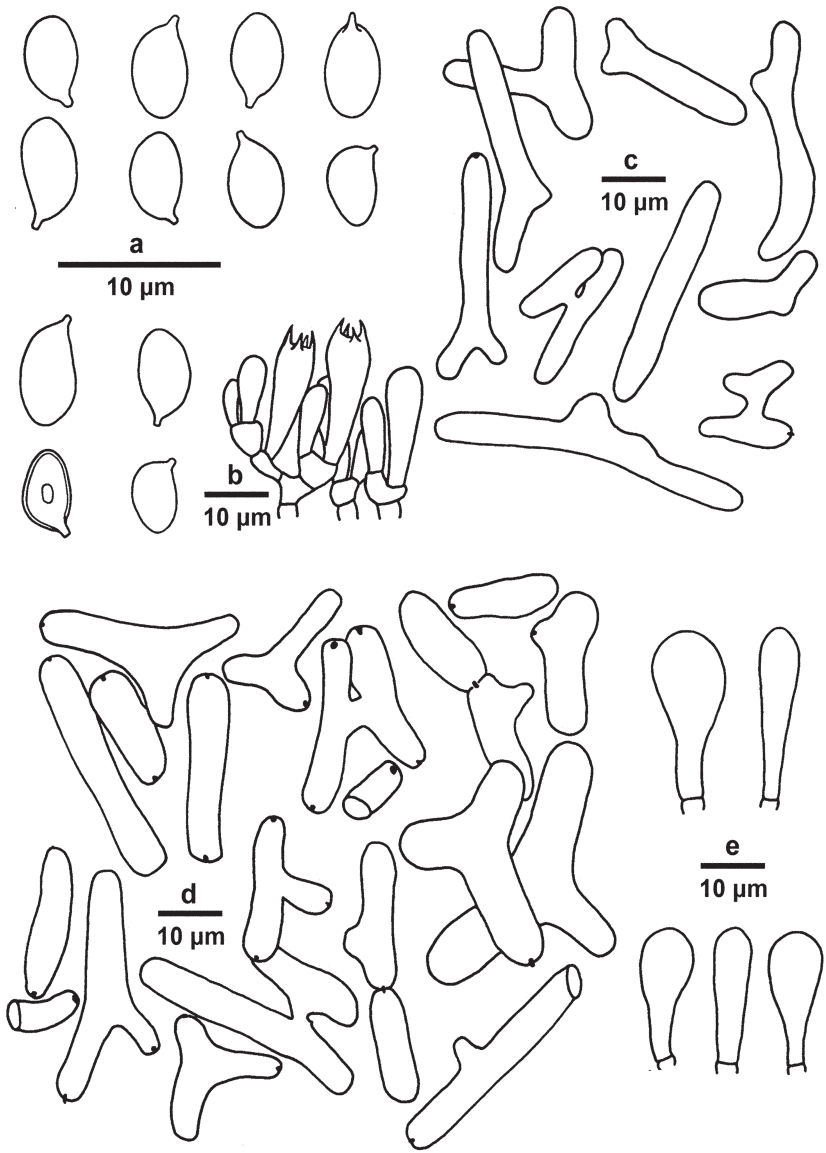

Figure 3. *Coniolepiota spongodes*. a. Basidiospores. b. Basidia at different stages of development; c. Stipe covering elements; d. Pileus covering elements; e. Cheilocystidia. (a = HKAS 77575; b–e = HKAS 77576).

Habit & Distribution — Solitary or in small groups of two along roadsides in forests of *S. robusta*, in Bangladesh. Known also from Sri Lanka, Thailand, Singapore, Malaysia, tropical China.

Specimens examined — **BANGLADESH. Rangpur Division**: Thakurgaon, Pirganj, Thumnia Sal Baghan, 70 m a.s.l., 18 July 2011, M.I. Hosen 239a (HKAS 77574; GenBank KC625530, KC625531); 18 June 2012, M.I. Hosen 373 (HKAS 77575); Dinajpur, Birganj, Singra Forest, 60 m a.s.l., 1 July 2013, M.I. Hosen 708 (SHAF 5); **Dhaka Division**: Tangail, Madhupur, Madupur National Park, 20 m a.s.l, 3 August 2012, M.I. Hosen 561 (HKAS 77576). **CHINA. Yunnan Province**: Luxi, Santai Mountain, 1200 m a.s.l., 29 July 1979, Zheng Wen Kang 79078 (HKAS 4848); Menhai, Mangao Nature Reserve, 1250 m a.s.l., 16 August 1991, Zhu L. Yang 1590 (HKAS 24071); Ruili, Nongdao, 1130 m a.s.l., 31 July 2003, Luo Hong 92 (HKAS 43631); **Hainan Province**: Wuzhishan, Wuzhishan Nature Reserve, 1867 m a.s.l., 1 August 2010, Z.W. Ge 2570 (HKAS 60246; GenBank KC871015).

Comments — *Coniolepiota spongodes* is restricted to southeast and south Asia. It is an attractive species for mushroom pickers as it can be readily recognized by the purplish to gray lilac coverings of the pileus and stipe surface. Distinguishing microscopic characters include the absence of abundant cheilocystidia, small smooth ellipsoid to oblong basidiospores, cylindrical elements in the pileal and stipe coverings, and absence of clamp connections in all tissues. Vellinga, who established *Coniolepiota* as an independent genus in *Agaricaceae* based on morphological and molecular evidence, presented the full synonymy of *C. spongodes* (Vellinga et al. 2011). According to Pegler (1972, 1986) basidiospores of *C. spongodes* are dextrinoid, which was confirmed by our study. The Chinese materials of *C. spongodes* also bear the same features that were observed in Bangladesh specimens: pileus and stipe coverings gray purple, basidiospores measuring [4/4/80] 4–5.5 × (2.5–)3–3.5(–4.0) µm [Q = 1.41–1.67, Q_m = 1.52 ± 0.118], ellipsoid, colorless, dextrinoid, inamyloid, slightly thick-walled, and without germ pore. Vellinga et al. (2011) reported that the basidiospores from Thai collections are pale or barely weakly dextrinoid in Melzer's reagent.

Phylogenetically, *C. spongodes* nests within the *Agaricus* s.l. clade based on multi-gene analysis with four different loci (ITS, nrLSU, tef1-α, and rpb2; Vellinga et al. 2011). *Coniolepiota spongodes* is closely related to the colored-spored genera *Agaricus* L., *Heinemannomyces* Watling, and *Clarkeinda* Kuntze (Vellinga et al. 2011). In our molecular analysis *H. splendidissima* Watling is strongly supported to be sister group to *Coniolepiota* with ML (96%) and BI (1.0) values, followed by *Agaricaceae* sp., *Eriocybe chionea* Vellinga and *Clarkeinda trachodes* (Berk.) Singer (Fig. 1). Vellinga et al. (2011) described the genus *Coniolepiota* to include collections of *C. spongodes* from Malaysia, Singapore, and Thailand. A collection from Thailand (ECV 3613), described as having pink-tinges to the pileus and stipe, was designated as *C.* aff. *spongodes* but not formally named. Minor differences in cheilocystidia shape and color of basidiomata were possibly due to environmental variation. Inclusion of our

Bangladesh and Chinese collections in phylogenetic analysis of ITS-nrLSU support the recognition of a single species in the genus rather than two distinct, sister species.

Acknowledgments

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